

What is claimed is:

- SUB
A1
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1. A vitamin K-dependent polypeptide comprising a modified GLA domain that enhances membrane binding affinity and activity of said polypeptide relative to a corresponding native vitamin K-dependent polypeptide, said modified GLA domain comprising at least one amino acid substitution, wherein said polypeptide is one that inhibits clot formation.
2. The polypeptide of claim 1, wherein said amino acid substitution is at amino acid 5, 9, 11, 12, 29, 33, 34, 35, or 36.
- SUB
A2
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3. The polypeptide of claim 1, wherein said amino acid substitution is at amino acid 5, 9, 35, or 36.
4. The polypeptide of claim 1, wherein said amino acid substitution is at amino acid 11 or 12.
- SUB
A2
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5. The polypeptide of claim 1, wherein said amino acid substitution is at amino acid 29 or 33.
6. The polypeptide of claim 1, wherein said amino acid substitution is at amino acid 34, 35, or 36.
7. The polypeptide of claim 1, wherein said polypeptide comprises Protein C or Activated Protein C.
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8. The polypeptide of claim 7, wherein said amino acid substitution comprises a glycine residue at amino acid 12.
9. The polypeptide of claim 7, wherein said amino acid substitution comprises a glutamic acid residue at amino acid 33.
10. The polypeptide of claim 8, wherein said amino acid substitution further comprises a glutamic acid residue at amino acid 33.
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11. The polypeptide of claim 9 or claim 10, wherein said amino acid substitution further comprises an aspartic acid or glutamic acid residue at amino acid 35.
12. The polypeptide of claim 9 or claim 10, wherein said amino acid substitution further comprises or a glutamic acid residue at amino acid 36.

13. The polypeptide of claim 9 or claim 10, wherein said amino acid substitution further comprises a glutamine or a glutamic acid residue at amino acid 11.

14. The polypeptide of claim 9 or claim 10, wherein said amino acid substitution further comprises a phenylalanine residue at amino acid 29.

5 15. The polypeptide of claim 9, claim 10, or claim 11, wherein said amino acid substitution further comprises an aspartic acid, glutamic acid, phenylalanine, leucine, or isoleucine residue at amino acid 34.

16. The polypeptide of claim 1, wherein said polypeptide comprises active-site modified Factor VIIa.

10 17. The polypeptide of claim 16, wherein said polypeptide comprises a glutamic acid at amino acid 33.

18. The polypeptide of claim 16, wherein said amino acid substitution comprises a glutamine residue at amino acid 11 and a glutamic acid residue at amino acid 33.

15 19. The polypeptide of claim 17 or claim 18, wherein said amino acid substitution further comprises a phenylalanine, leucine, or isoleucine residue at amino acid 34.

20 20. The polypeptide of claim 17, claim 18, or claim 19, wherein said amino acid substitution further comprises an aspartic acid or a glutamic acid residue at amino acid 35.

21. The polypeptide of claim 1, wherein said polypeptide is Protein S.

22. The polypeptide of claim 21, wherein said amino acid substitution comprises an isoleucine, leucine, valine, or phenylalanine residue at amino acid 9.

25 23. The polypeptide of claim 22, wherein said amino acid substitution further comprises a phenylalanine, leucine, isoleucine, aspartic acid, or glutamic acid residue at amino acid 34 or an aspartic acid or glutamic acid residue at amino acid 35.

24. The polypeptide of claim 21, wherein said amino acid substitution comprises a phenylalanine residue at amino acid 5.

30 25. The polypeptide of claim 21, wherein said polypeptide further comprises a substitution in the thrombin-sensitive loop.

26. The polypeptide of claim 25, wherein said substitution in the thrombin sensitive loop is at amino acid 49, 60, or 70.

27. The polypeptide of claim 1, wherein said polypeptide is active-site modified Factor IXa.

5 28. The polypeptide of claim 27, wherein said amino acid substitution comprises a phenylalanine at amino acid 29.

29. The polypeptide of claim 27, wherein said amino acid substitution comprises a phenylalanine residue at amino acid 5.

10 30. The polypeptide of claim 27, wherein said amino acid substitution comprises a phenylalanine, leucine, or isoleucine at amino acid 34 and an aspartic acid or glutamic acid residue at amino acid 35.

31. The polypeptide of claim 1, wherein said vitamin K-dependent polypeptide further comprises an inactivated cleavage site.

15 32. The polypeptide of claim 31, wherein said polypeptide comprises factor VII.

33. The polypeptide of claim 32, wherein said inactivated cleavage site comprises a substitution of an alanine residue at amino acid 152.

20 34. The polypeptide of claim 1, wherein said polypeptide is active site modified factor Xa.

35. The polypeptide of claim 33, wherein said amino acid substitution comprises a glutamine at amino acid 11.

36. The polypeptide of claim 34, wherein said amino acid substitution comprises a phenylalanine, leucine, or isoleucine at amino acid 34 and an aspartic acid or glutamic acid residue at amino acid 35.

25 37. The polypeptide of claim 1, wherein said polypeptide is protein Z.

38. The polypeptide of claim 37, wherein said amino acid substitution comprises a phenylalanine, leucine, or isoleucine residue at amino acid 34, or an aspartic acid or glutamic acid residue at amino acid 35.

30 39. A vitamin K-dependent polypeptide comprising a modified GLA domain that enhances membrane binding affinity and activity of said polypeptide

relative to a corresponding native vitamin K-dependent polypeptide, said modified GLA domain comprising at least one amino acid insertion at amino acid 4.

40. The polypeptide of claim 39, wherein said polypeptide is selected from the group consisting of factor VII or factor VIIa, protein C or activated protein C,
5 factor X or factor Xa, and protein S.

41. The polypeptide of claim 39, wherein said polypeptide is factor VII, factor VIIa, protein C, or activated protein C.

42. The polypeptide of claim 41, wherein said amino acid insertion comprises a tyrosine residue.

10 43. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective amount of a vitamin K-dependent polypeptide, wherein said vitamin K-dependent polypeptide comprises a modified GLA domain that enhances membrane binding affinity and activity of said polypeptide relative to a corresponding native vitamin K-dependent polypeptide, said modified GLA domain
15 comprising at least one amino acid substitution, and wherein said vitamin K-dependent polypeptide inhibits clot formation.

44. The pharmaceutical composition of claim 43, wherein said polypeptide is Protein C or Activated Protein C.

45. The pharmaceutical composition of claim 44, wherein said amino acid
20 substitution comprises a glycine at amino acid 12.

46. The pharmaceutical composition of claim 45, wherein said amino acid substitution further comprises a glutamic acid residue at amino acid 33.

47. The pharmaceutical composition of claim 45, wherein said amino acid substitution further comprises a phenylalanine, leucine, or isoleucine residue at amino
25 acid 34, and an aspartic acid or glutamic acid residue at amino acids 35 or 36.

48. The pharmaceutical composition of claim 43, wherein said polypeptide is active-site modified Factor VIIa.

49. The pharmaceutical composition of claim 48, wherein said amino acid substitution comprises a glutamine residue at amino acid 11, a glutamic acid residue
30 at amino acid 33, a phenylalanine, leucine, or isoleucine residue at amino acid 34, and an aspartic acid or glutamic acid residue at amino acid 35.

50. The pharmaceutical composition of claim 43, wherein said polypeptide is Protein S or active-site modified Factor IXa.

51. The pharmaceutical composition of claim 43, wherein said composition further comprises an anticoagulant agent.

52. A mammalian host cell comprising a vitamin K-dependent polypeptide, said vitamin K-dependent comprising a modified GLA domain that enhances membrane binding affinity and activity of said polypeptide relative to a corresponding native vitamin K-dependent polypeptide, said modified GLA domain comprising at least one amino acid substitution, wherein said polypeptide is one that inhibits clot formation.

53. A method of decreasing clot formation in a mammal comprising administering an amount of a vitamin K-dependent polypeptide effective to decrease clot formation in said mammal, wherein said vitamin K-dependent polypeptide comprises a modified GLA domain that enhances membrane binding affinity and activity of said polypeptide relative to a corresponding native vitamin K-dependent polypeptide, said modified GLA domain comprising at least one amino acid substitution.

54. The method of claim 53, wherein said polypeptide is Protein C or Activated protein C, active-site modified Factor VIIa, active-site modified Factor IXa, or Protein S.

55. A method for identifying a vitamin K-dependent polypeptide having enhanced membrane binding affinity and activity comprising:

(a) modifying the GLA domain of said vitamin K-dependent polypeptide, wherein said modifying comprises substituting at least one amino acid in said GLA domain;

b) monitoring membrane binding affinity and activity of said vitamin K-dependent polypeptide having said modified GLA domain; and

(c) identifying said modified vitamin K-dependent polypeptide as having enhanced membrane binding affinity and activity if membrane binding affinity and activity of said modified vitamin K-dependent polypeptide is enhanced relative to a corresponding native vitamin K-dependent polypeptide.

56. The method of claim 55, wherein said amino acid substitution is at amino acid 5, 9, 11, 12, 29, 33, 34, 35, or 36.

57. The method of claim 55, wherein said modified vitamin K-dependent polypeptide increases clot formation.

5 58. The method of claim 55, wherein said modified vitamin K-dependent polypeptide inhibits clot formation.

59. A Factor VII or Factor IX polypeptide comprising a modified GLA domain that enhances membrane binding affinity of said polypeptide relative to a corresponding native Factor VII or Factor IX polypeptide, said modified GLA domain comprising at least one amino acid substitution at residue 11, 29, 34, or 35.

60. The polypeptide of claim 59, wherein said polypeptide comprises Factor VII or Factor VIIa.

61. The polypeptide of claim 60, wherein a glutamine, a glutamic acid, an aspartic acid, or an asparagine residue is substituted at amino acid 11.

62. The polypeptide of claim 60, wherein a glutamine residue is substituted at amino acid 11.

63. The polypeptide of claim 59 or claim 60, wherein a glutamic acid or a phenylalanine residue is substituted at amino acid 29.

64. The polypeptide of claim 60, wherein said modified domain further comprises an amino acid substitution at amino acid 33.

65. The polypeptide of claim 64, wherein a glutamic acid residue is substituted at amino acid 33.

66. The polypeptide of claim 61 or claim 63, wherein said modified GLA domain further comprises a substitution of a glutamic acid residue at amino acid 33.

67. The polypeptide of claim 60, wherein said modified GLA domain further comprises at least one hydrophobic residue at residue 34 or 35.

68. The polypeptide of claim 60, wherein said modified GLA domain further comprises a phenylalanine, leucine, or isoleucine residue at amino acid 34 and an aspartic acid or glutamic acid residue at amino acid 35.

69. The polypeptide of claim 59, wherein said polypeptide comprises Factor IX or Factor IXa.

70. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an amount of a Factor VII or Factor IX polypeptide effective to increase clot formation in a mammal, wherein said Factor VII or Factor IX polypeptide comprises a modified GLA domain that enhances membrane binding affinity of said polypeptide relative to a corresponding native Factor VII or Factor IX polypeptide, said modified GLA domain comprising at least one amino acid substitution at residue 11, 29, 34, or 35.

71. The pharmaceutical composition of claim 70, wherein said polypeptide further comprises an amino acid substitution at amino acid 33.

72. The pharmaceutical composition of claim 70, wherein said pharmaceutical composition further comprises soluble tissue factor.

73. A method of increasing clot formation in a mammal comprising administering an amount of a Factor VII or Factor IX polypeptide effective to increase clot formation in said mammal, wherein said Factor VII or Factor IX polypeptide comprises a modified GLA domain that enhances membrane binding affinity of said polypeptide relative to a corresponding native Factor VII or Factor IX polypeptide, said modified GLA domain comprising at least one amino acid substitution at residue 11, 29, 34, or 35.

74. A method for treating a bleeding disorder in a patient, said method comprising administering the pharmaceutical composition of claim 73 to said patient.

75. An isolated nucleic acid molecule, said molecule comprising a nucleic acid sequence encoding the polypeptide of claim 1 or claim 59.

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